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SHORT COMMUNICATIONS

Ir GENE CONTROL OF T AND B CELL RESPONSES TO DETERMINANTS IN (Glu Lys Ala) TERPOLYMERS

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SUMMARY

All mice responding to the terpolymer GLA^{40} make GL, GA and GLA specific antibodies irrespective of their response to GL or GA alone. The mice displayed positive T cell proliferative responses against the homologous terpolymer, but no T cell responses were obtained with GL, which is non-immunogenic in mice. T cells from GLA immune mice, which are also responders to GA, such as mice of *H-2* haplotypes *a*, *b*, *d*, *k* and *r*, could be stimulated by GA. T cells from GLA immune mice of *H-2* haplotypes *p* and *q* which are non-responders to GA could not be stimulated by GA. On the other hand, T cells from *H-2*^{s} mice immune to GLA and which are also responders to GA alone could not be stimulated by GA. Thus mice of *H-2* haplotypes *p*, *q* and *s* recognize the terpolymer via 'GLA' determinants alone, whereas mice of *H-2* haplotypes *a*, *b*, *d*, *k* and *r* may recognize both GA and GLA determinants in GLA terpolymer.

An important question in immunobiology today is to delineate whether all animals of any species injected with a multi-determinant immunogen recognize such a macromolecule via the same or different determinants. Previously we have shown that specific immune responses to the random linear terpolymers containing varying amounts of the amino acids L-glutamic acid, L-lysine and L-alanine (GLA) are controlled by immune response (*Ir*) genes linked to the major histocompatibility locus in mice (Maurer & Merryman, 1974a; Maurer & Merryman, 1978). It was shown that mice immunized with these GLA polymers made anti-GLA antibodies and antibodies cross-reacting with GA and GL in differing amounts (Maurer & Merryman, 1978).

The data in Table 1 show that the strains of mice in this study made varying percentages of GA and GL specific plaques when immunized with GLA^{40}. The number of GLA specific plaques was always higher than the number of GL specific plaques. This finding is in contrast to those reported by Cheung *et al.* (1978). In their studies they employed palmitoyl conjugates of GLA^{10} and/or GL to couple the polymer to SRBC, and found the

TABLE 1. Antibody cross-reactions (GA and GL) of mice in response to 100 μg of GLA^{40}

Strain	*H-2* haplotype	Inhibitable (%) PFC per spleen			Specific antigen (%) bound with:		
		GLA^{40}	GA	GL	GLA^{40}	GA	GL
A/He	*a*	100	70	40	83	82	45
C57BL/10	*b*	100	56	30	83	76	55
B10.M	*f*	100	49	42	88	87	53
B10.Q	*q*	100	50	40	45	49	42
RIII	*r*	100	74	30	69	71	48
SJL	*s*	100	30	25	66	57	62

Mice were immunized with 100 μg of GLA^{40}. They were boosted on day 21 with 10 μg of GLA^{40} except A/He, B10.M and B10.Q which were boosted with 100 μg of GLA^{40}. The values are from a pool of three mice per group. Fifty micrograms of GLA^{40} or GA or GL is added to check the specific PFC.

same number of plaques with either GL or GLA^{10} coated SRBC. In our studies we have coupled the GLA polymers directly to SRBC by the tannic acid procedure as described by Baltz *et al.* (1978) and detected the plaques against the homologous terpolymers as well as against cross-reacting copolymers.

The data in Table 2 show that all responding mice elicited positive T cell proliferative responses against the homologous terpolymer but no T cell responses were detected with GL, which is known to be non-immunogenic in all the mice studied so far. In contrast to this, the above T cells from mice responding to GLA^{40} showed at least three patterns when the proliferative responses were measured with GA. (1) Mice of haplotypes *a*, *b*, *d*, *k* and *r* that can respond to GA when used as an immunogen exhibited cross T cell proliferative responses with GA; (2) Mice of *H-2* *p*, *q*, and *s* haplotypes did not respond to GA. These three 'non-responders' to GA can be divided further as follows; (3) The mice of *H-2* *p* and *q* haplotypes do not respond to GA as an immunogen, whereas mice of the $H\text{-}2^s$ haplotype do respond to GA as an immunogen, but not following immunization with the GLA

TABLE 2. T cell proliferative responses with GLA^{40} and GA from mice immunized with 100 μg of GLA^{40}

Strain	*H-2* haplotype	Background cpm	Stimulation index with: GLA^{40}	GA
A/He	*a*	1,950	11	4
C57BL/10	*b*	520	18	7
BALB/c	*d*	630	23	9
C3H	*k*	560	22	13
P/J	*p*	870	10	1
DBA/1	*q*	1,370	8	1
RIII	*r*	990	16	4
SJL	*s*	400	94	2

Stimulation Index values refer to cultures stimulated with an optimal concentration (100 μg/ml) of GLA^{40} or GA.

terpolymers. These findings support the concept that there are differences in the specificities and also the repertoire of the T cells recognizing the GLA^{40}, i.e., mice of *H-2 p* and *q* haplotypes recognize the terpolymer via GLA determinants alone, whereas mice of *H-2 a, b, d, k* and *r* haplotypes may recognize both GA and GLA determinants at the T cell level and produce clones of educated T cells having those specificities. This conclusion is reinforced by the responses of mice of the $H\text{-}2^s$ haplotype that ordinarily respond well to GA, do have T cells with receptors for GA, but in the experiments reported here respond only by recognition of GLA determinants.

The absence of GL and GA proliferative T cell responses in mice of *H-2 p* and *q* haplotypes following immunization with GLA is in agreement with the proposal by Schwartz (1975) that ordinarily only immunogenic determinants can stimulate a T cell proliferative response, either homologous or heterologous. In a separate publication the experiments dealing with the non-responsiveness of mice of the $H\text{-}2^s$ haplotype to GA will be presented.

That mice of the $H\text{-}2^q$ haplotype are unique in their recognition of GLA determinants in the random terpolymers of GLA is clearly shown by their positive immune responses to GLA^5, negative responses to GLA^{10} and GLA^{20} and positive responses to GLA^{40} and GLA^{60}, but only following immunization with 100 μg of the terpolymers. Similarly, mice of the $H\text{-}2^b$ haplotype, which are non-responders to GLA^5, do exhibit increasing reactivity against the terpolymers GLA^{10}, GLA^{20}, GLA^{40} and GLA^{60} (Maurer & Merryman, 1978). These latter mice also respond to GA.

An important conclusion of these findings is that mice of different haplotypes with varying Ia specificities can indeed recognize different determinants present within the same complex random immunogen. This conclusion is in agreement with the findings of others and our laboratory dealing with $GLPhe^9$ (Baltz *et al.*, 1978; Maurer & Merryman, 1978).

A comparison of levels of the antibody produced by inbred strains and their congenic inbred partners showed clearly that non-*H-2* genes have a marked effect on the magnitude

TABLE 3. Influence of B10 background in mice in response to 100 μg of GLA^{40}

		1°, 21 days		2°, 7 days	
Strain	*H-2* haplotype	Total PFC/10^6 cells	Antigen bound (%)	Total PFC/10^6	Antigen bound (%)
A/WySn	*a*	52 ± 13	80 ± 7	240 ± 22	83 ± 6
B10.A	*a*	5 ± 1	65 ± 6	100 ± 9	67 ± 8
DBA/2	*d*	28 ± 4	47 ± 6	122 + 12	61 ± 6
B10.D2	*d*	15 ± 3	39 ± 3	28 ± 6	52 ± 5
P/J	*p*	5 ± 2	8 ± 2	54 ± 11	29 ± 10
B10.P	*p*	0	0	14 ± 3	19 ± 6
DBA/1	*q*	20 ± 4	31 ± 7	400 ± 130	76 ± 23
B10.Q	*q*	9 ± 3	19 ± 5	190 ± 35	43 ± 16
RIII	*r*	10 ± 2	51 ± 15	52 ± 12	69 ± 18
B10.RIII	*r*	5 ± 2	27 ± 6	28 ± 10	53 ± 9
SJL*	*s*	67 ± 6	48 ± 7	470 ± 215	83 ± 8
A.SW	*s*	20 ± 3	39 ± 4	960 ± 110	54 ± 6
B10.S	*s*	8 ± 2	23 ± 6	480 ± 75	38 ± 7

Conditions as for Table 1. *SJL mice were boosted with 10 μg of GLA^{40}.

of antibody responses to the terpolymer GLA[40]. As can be seen in Table 3 the B10 background clearly lowered the magnitude of response as measured by both the antigen binding assay and the PFC assay. Similar observations of the influence of non-*H-2* genes on the magnitude of immune response have been observed for GLA[10] (Maurer & Merryman, 1974b) and for responses against other immunogens such as SRBC (Ando & Fachet, 1977).

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